E

After injection of transgenic SMARTA splenocytes, sustained CD4+ T-cell activity was detected in three recipient animals. Viruses subsequently emerged which carried mutations within the GP61-80 epitope. As expected, only wild-type GP61-80 sequences were detected in mice without sustained CD4+ T-cell activity after splenocyte transfer.

The results of Ciurea *et al.* clearly document a directional change in antigenic sequence over time in a targeted CD4+ T-cell epitope under experimental conditions. Variants completely replaced the wild-type viral population, and were not recognized by the sustained immunodominant CD4+ T-cell response. Can CD4+ T cells therefore select viral immune escape variants?

The preferential targets for LCMV infection are APCs that express MHC class II molecules. Direct CD4+ T-cell–mediated antiviral effector mechanisms could therefore specifically target infected cells. However, no MHC class II-restricted cytotoxicity could be detected in the recipient mice after adoptive transfer of SMARTA splenocytes. Other potential antiviral mechanisms, such as cytokine release, might therefore account for the selection force exerted on the viral population by CD4+ T cells. In this respect, it would be interesting to know the extent to which SMARTA splenocytes affect viral replication in recipient animals[8].

It is more difficult to envisage how selection might operate if CD4+ T cells act indirectly unless common antigenic determinants are invoked. Although T-cell tolerance in B6 mice neonatally infected with LCMV is absolute, activation of virus-reactive B cells is prevented primarily by a lack of specific CD4+ T-cell help[9]. Therefore, adoptive transfer of LCMV-specific CD4+ T cells could potentially induce a host antibody response to free virus or virus-derived proteins, such as GP-1, that are expressed on the surface of infected cells. Such antibodies could drive the positive selection of mutants in targeted epitopes. Although a formal analysis of genetic selection is not presented, the full-length GP-1 sequence in one of the three mice studied in detail after injection of SMARTA splenocytes failed to reveal additional mutations outside the CD4+ T-cell epitope region. This excludes the possibility that the GP75 mutation in this case was a compensatory change linked to mutations elsewhere selected by distinct immune forces[10]. However, the possibility of selection mediated by neutralizing antibodies directed against an epitope containing the GP75 residue remains.

In summary, Ciurea *et al.* elegantly demonstrate that escape mutations in a dominant CD4+ T-cell epitope can replace original antigenic sequences within a RNA virus population during experimental persistent infection. The challenges for future research in the field will be to define the mechanism through which this occurs, and to determine the biological relevance of these findings for chronic infections with major pathogens such as HIV and hepatitis C virus.

1. Pircher, H. *et al.* Viral escape by selection of cytotoxic T cell-resistant virus variants *in vivo*. *Nature* **346**, 629–633 (1990).
2. Ciurea, A. *et al.* CD4+ T-cell–epitope escape mutant virus selected in vivo. *Nature Med.* **7**, 795-800 (2001).
3. Holland, J. *et al.* Rapid evolution of RNA genomes. *Science* **215**, 1577–1585 (1982).
4. Sewell, A.K., Price, D.A., Oxenius, A., Kelleher, A.D. & Phillips, R.E. Cytotoxic T lymphocyte responses to human immunodeficiency virus: Control and escape. *Stem Cells* **18**, 230–244 (2000).
5. Lanzavecchia, A. Immunology. Licence to kill. *Nature* **393**, 413–414 (1998).
6. Ciurea, A. *et al.* Viral persistence in vivo through selection of neutralizing antibody-escape variants. *Proc Natl Acad Sci USA* **97**, 2749–2754 (2000).
7. Harcourt, G.C., Garrard, S., Davenport, M.P., Edwards, A. & Phillips, R.E. HIV-1 variation diminishes CD4 T lymphocyte recognition. *J. Exp. Med.* **188**, 1785–1793 (1998).
8. Oxenius, A., Bachmann, M.F., Zinkernagel, R.M. & Hengartner, H. Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. *Eur. J. Immunol.* **28**, 390–400 (1998).
9. Seiler, P. *et al. In vivo* selection of neutralization-resistant virus variants but no evidence of B cell tolerance in lymphocytic choriomeningitis virus carrier mice expressing a transgenic virus-neutralizing antibody. *J. Immunol.* **162**, 4536–4541 (1999).
10. Kelleher, A.D. *et al.* Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27–restricted cytotoxic T lymphocyte responses. *J. Exp. Med.* **193**, 375–386 (2001).

Nuffield Department of Clinical Medicine
John Radcliffe Hospital
Oxford, UK
Email: rodney.phillips@ndm.ox.ac.uk

Protein-based PCR for prion diseases?

The development of a sensitive *in vitro* method to amplify the pathological form of the prion protein might provide a tool for the pre-clinical diagnosis of prion diseases.

GLENN TELLING

The transmissible spongiform encephalopathies (TSEs) are a group of closely related neurodegenerative conditions of animals and humans caused by prions[1]. Their extraordinary biology and the unique properties of the infectious agent attracted interest and considerable controversy well before the epidemic of bovine spongiform encephalopathy (BSE) and the subsequent appearance of a new variant of Creutzfeldt–Jakob disease (vCJD) in humans. The death toll of vCJD is currently over 100, with the future number of cases an uncertainty. Compelling evidence that vCJD is the human manifestation of BSE (ref. 2) brings into question the risk to humans from other prion diseases such as chronic wasting disease (CWD) of deer and elk. Thus, a rapid and sensitive diagnostic test to specifically diagnose prion disease in pre-symptomatic animals and humans is of the utmost importance. In the 14 June issue of *Nature*, Saborio *et al.*[3] report the development of a sensitive *in vitro* method to detect the pathological form of the prion protein using a novel amplification technique, which, if issues of specificity can be satisfactorily addressed, will have enormous impact as a diagnostic tool for prion diseases.

Currently available methods to diagnose prion diseases rely either on prion infectivity assays in susceptible hosts or on the immunodetection of the pathogenic form of the prion protein (PrP). Infectivity assays are the most definitive and might be sensitive enough to detect prions at levels as low as one infectious unit. However, the time between inoculation and disease is long, particularly at low prion titers—a factor that obviously precludes rapid diagnosis. On the other hand, detection of pathogenic PrP is limited by the amount of abnormal protein in affected tissues. This means that detection is usually achieved by analyzing brain tissue at autopsy or, in some cases, tonsil biopsies in the late stages of disease, after the prions have wreaked havoc and potentially transmitted to other hosts.

To improve the sensitivity of current detection methods, several groups are attempting to develop assays that exploit the conversion of normal prion protein to the pathogenic form. The normal form, PrP^{C}, is a 33–35-kD sialoglycoprotein attached to the cell surface by a glycophoshphatidyl in-

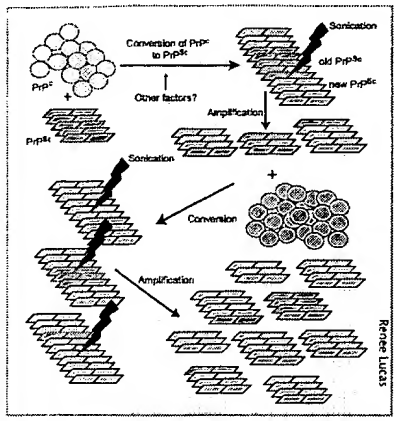

Fig. 1 Diagrammatic representation of protein misfolding cyclic amplification (PMCA), adapted from Saborio *et al.* PrP^C is recruited into growing aggregates of PrP^{Sc} where it undergoes conformational conversion and acquires properties associated with PrP^{Sc}. During PMCA, the growing PrP^{Sc} oligomer is disrupted by repeated sonication in the presence of detergents. This treatment generates an expanded population of converting units for the continued recruitment of PrP^C. PrP^C (α-helical conformation) is shown as light yellow spheres. PrP^{Sc} (β-sheet conformation) is shown as rhombuses. The original seed is in dark yellow and the newly converted PrP is in light yellow.

ositol (GPI) anchor. It is sensitive to proteases, soluble in detergents and has a high α-helical content. The disease-associated isoform, PrP^{Sc}, has high β-sheet content, is partially resistant to proteases, insoluble in detergents and tends to aggregate. Protease cleavage of approximately 66 of the N-terminal amino acids of PrP^{Sc} gives rise to a protease-resistant core referred to as PrP27–30 or PrP^{Res}. Considerable evidence argues that misfolded prions are composed largely, if not exclusively, of PrP^{Sc} and that the central event in prion replication is the coercion of PrP^C by PrP^{Sc} to adopt the infectivity-associated conformation.

Previous attempts to replicate PrP conversion in cell-free systems consisted of prolonged incubation of radiolabeled PrP^C with equimolar amounts of partially denatured PrP^{Sc} (ref. 4). Using an approach called protein-misfolding cyclic amplification (PMCA), Saborio *et al.* now show that much smaller amounts of hamster PrP^{Sc} can rapidly convert PrP^C in uninfected hamster brain preparations into protease-resistant PrP. During PMCA, disruption of the growing oligomer of PrP^{Sc} is accomplished by sonication in the presence of detergents—a method routinely used to solubilize PrP27–30 (ref. 5). This process generates multiple smaller templates for the continued recruitment of PrP^C, which undergoes conformational conversion and acquires properties associated with PrP^{Sc} (Fig. 1). As PrP^C is distinguished from PrP^{Sc} based on differential protease sensitivity, it is possible to monitor amplification of the PrP^{Sc} signal following protease treatment and immunoblotting. After five cycles of incubation–sonication, the newly converted protein accounted for approximately 98% of protease resistant PrP and this ratio could be further increased by additional cycles. The technique was sensitive enough to detect approximately 6–12 pg or $0.2–0.4 \times 10^{-15}$ mol of PrP^{Sc} after a 10,000-fold dilution of infected hamster brain homogenate and 10 cycles of PMCA.

As they stand, these results raise some important questions about the specificity of the conversion reaction, particularly in relation to the species-barrier phenomena associated with prion biogenesis *in vivo*[6]. Although the authors showed that levels of hamster PrP^{Sc} do not increase when added to uninfected rat brain homogenates, they did not measure total PrP^{Sc} using antibodies that would detect rat PrP. The most convincing demonstration that specificity is maintained in this system would be to show that PrP^{Sc} from another species (such as mouse, sheep or human) fails to convert hamster PrP^C by PMCA. The propagation of pathogenic prion proteins by different hamster strains could also be easily monitored based on different molecular masses of PrP27-30 (ref. 7).

The results of infectivity assays are also eagerly awaited as they will have important implications for understanding the mechanism of prion propagation. In previous *in vitro* systems, the stoichiometric excess of infectious PrP^{Sc} used to drive the conversion reaction precluded the detection of newly synthesized prions *in vitro*. Because the input level of PrP^{Sc} in the PMCA assay is relatively small, it should be possible to detect an increased titer of infectious prions after amplification, assuming that the protease-resistant PrP produced by the *in vitro* reaction retains the infectious character of PrP^{Sc} produced *in vivo*.

In addition to their diagnosistic relevance, the results presented in this report have implications for the identification of putative factor(s) that facilitate conversion of PrP^C to PrP^{Sc} *in vivo*. Even in the absence of PMCA the authors were able to detect a significant increase in protease-resistant PrP after the addition of PrP^{Sc} to uninfected hamster brain preparations—an effect that was not seen in previous studies using purified proteins or cell lysates. The authors ascribe this effect to the presence of catalytic factor(s) in the brain homogenate that would presumably have the same or similar functions *in vivo* as PMCA. This suggestion is not unreasonable given that the existence of such factors has been postulated from studies in transgenic mice[8]. PMCA provides a convenient assay that will facilitate purification of converting activity from brain preparations.

Finally, these data raise a note of caution. The extreme sensitivity of this technique demonstrates that methods employing sonication of brain tissues to prepare PrP^{Sc} are potentially subject to false-positive values resulting from cross contamination with PrP^{Sc}.

If the issues regarding specificity can be satisfactorily addressed, PMCA should provide a means of detecting extremely small amounts of PrP^{Sc} in systemic tissues or body fluids and become an important tool for the pre-clinical diagnosis of prion disease. Using PMCA, it might be possible to test for the presence of prions in blood during the early phase of disease. This would not only ensure that all animals were tested for prion disease before they entered the food chain, but would also be a huge step toward ensuring that the human blood supply remains uncontaminated. PMCA might also help to facilitate our understanding of the molecular events involved in prion replication and will likely be extremely useful for identifying compounds that inhibit prion replication.

1. Prusiner, S.B. Prions. *Proc. Natl. Acad. Sci. USA* **95**, 13363–13383 (1998).
2. Collinge, J. Variant Creutzfeldt-Jakob disease. *Lancet* **354**, 317–323 (1999).
3. Saborio, G.P., Permanne, B. & Soto, C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411**, 810–813 (2001).
4. Kocisko, D.A. *et al.* Cell-free formation of protease-resistant prion protein. *Nature*, **370**, 471–474 (1994).
5. Riesner, D. *et al.* Disruption of prion rods generates 10-nm spherical particles having high α-helical content and lacking scrapie infectivity. *J. Virol.* **70**, 1714–1722 (1996).
6. Scott, M. *et al.* Transgenic mice expressing hamster prion protein produce species-specific scrapie infectivity and amyloid plaques. *Cell* **59**, 847–857 (1989).
7. Bessen, R.A. *et al.* Non-genetic propagation of strain-specific properties of scrapie prion protein. *Nature* **375**, 698–700 (1995).
8. Telling, G.C. *et al.* Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* **83**, 79–90 (1995).

Department of Microbiology and Immunology,
Department of Neurology and the
Sanders Brown Center on Aging
University of Kentucky
Lexington, Kentucky, USA
Email: gtell2@pop.uky.edu